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Effects of Ambient Flow and Injury on the Morphology of a Fluid Transport System in a Bryozoan

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Abstract. Many organisms use fluid transport systems that are open to the external environment for suspension feeding or gas exchange. How do factors related to the environment, such as injuries and ambient currents, affect remodeling of these systems? In the bryozoan *Membranipora membranacea,* the lophophores (crowns of ciliated tentacles) form a canopy over the colony. The lophophores pump seawater from above the colony through themselves to capture food particles. The seawater then flows under the canopy to exit the colony at chimneys (openings in the canopy) or at the canopy edge. To test whether either ambient flow speed or injury affects remodeling of this system, I measured changes in chimney size and spacing in colonies grown in flow tanks at different ambient flow speeds, and in colonies in which I killed patches of zooids. There was no effect of either ambient flow speed or injury size on chimney remodeling. Injury did not induce chimney formation. In addition, chimneys formed at the canopy edge, indicating that high pressure under the canopy did not induce chimney formation. These results suggest that ambient flow, injury, and the pressure under the canopy may have little effect on the remodeling of this fluid transport system.

Introduction

Systems in which organisms pump fluids (*e.g.,* blood, water) through themselves serve a variety of major functions including internal transport, respiration, and suspension feeding (LaBarbera, 1990). These fluid transport systems share common physical and functional principles (LaBarbera and Vogel, 1982; LaBarbera, 1990, 1995). For example, resistance to flow is greater in narrow vessels than in wide vessels; however, there is frequently a cost to building wide vessels. Therefore, vessel size tends to increase as the flow rate through the vessel increases (LaBarbera, 1990).

Many internal fluid transport systems remodel in response to changes in the flow through the system. Several studies have shown that blood vessels in the mammalian circulatory system remodel in response to changes in the flow through them (reviewed in LaBarbera, 1990, 1995; Langille, 1995). Other studies suggest that changes in flow induce remodeling in the gastrovascular canals of hydroid colonies (Dudgeon and Buss, 1996; Buss, 2001) and the veins of plasmodial slime molds (Nakagaki *et al.,* 2000, 2001). These systems all pump fluid through pipe-like conduits that are isolated from the external environment.

Organisms also use fluid transport systems for suspension feeding or respiration (LaBarbera, 1990). In contrast to internal fluid transport systems, these systems interact with the ambient flow environment through conduits that form openings onto the external fluid. These conduits are used either to take in unprocessed fluid or to expel processed fluid. They include the siphons of ascidians and clams, and the oscula of sponges. In many bryozoans that form sheet-like colonies (Banta *et al.,* 1974; Cook, 1977; Winston, 1979), and in some colonial ascidians, several individuals pump filtered seawater through the colony to exit at common excurrent openings (chimneys). Certain large, sulfur-oxidizing bacteria even form chimney-like structures that are important for maintaining proper O_2 concentrations (Fenchel and Glud, 1998). Can conduits that connect with the ambient flow environment remodel, and if so, what factors affect their remodeling?

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Chimneys in the bryozoan Membranipora membranacea

The bryozoan *Membranipora membranacea* Linnaeus, 1767, is an excellent system with which to study the effects of flow on fluid transport systems involved in suspension feeding because *M. membranacea* colonies grow rapidly and form a simple fluid transport system. The colonies are composed of a sheet of physiologically connected individuals (zooids) bearing lophophores (crowns of ciliated tentacles) that form a canopy over most of the colony (Fig 1A–D). Groups of lophophores lean away from each other to form openings called chimneys (Fig. 1; Banta *et al.*, 1974; Lidgard, 1981). Frequently, several zooids in the center of a chimney do not extend their lophophores and do not feed (Lidgard, 1981). The lophophores capture food particles from seawater that they pump from above the colony down towards the colony and between the tentacles. The seawater then flows under the canopy of closely packed lophophores to exit the colony at the canopy edge or at one of the chimneys (Fig. 1B; Banta *et al.*, 1974; Lidgard, 1981).

Previous studies have suggested that flow around and through the colony affects where new chimneys are formed in *M. membranacea* (Dick, 1987; Grünbaum, 1997; Okamura and Partridge, 1999). Okamura and Partridge (1999) found that chimney spacing decreased with increasing ambient flow speed in the field. Grünbaum (1997) found that chimney spacing was reduced in colonies with spines, an inducible defense against specific nudibranch predators, and that chimney shape depended on the shape of the substratum on which colonies were grown. Since both the presence of spines and the shape of the substratum (which determines the shape of the colony) were predicted to affect the flow through the colony, these results suggested a hydromechanical mechanism of chimney formation (Grünbaum, 1997). However, none of these studies evaluated whether chimneys could remodel after they had formed.

Remodeling of this system could result from changing the extension or orientation of lophophores, from degeneration of feeding zooids, or from regeneration of nonfeeding zooids. These processes could potentially result in new chimneys forming within the canopy as suggested by Dick (1987), or in changes in the size or position of existing chimneys. Alternatively, new chimneys could form at the canopy edge since the colony grows by addition of new zooids at the edge of the colony (Dick, 1987).

What factors might affect the remodeling of existing chimneys?

Fluid flow through and around a colony could change over time due to changes in the ambient flow speed or to injury to the colony. These two factors are likely to be important in the environment given the variable flow conditions in which these colonies grow (Okamura and Partridge, 1999) and the presence of predators that injure colonies (Yoshioka, 1982; Harvell, 1984). Ambient flow speed is known to affect the feeding performance of other sheet-like bryozoans (Okamura, 1985), as well as both the rate (Eckman and Duggins, 1993; Grünbaum, 1997) and direction (Norton, 1973) of growth in *M. membranacea*.

Ambient flow can generate passive flow through a structure. In some active suspension feeders such as sponges (Vogel, 1977) and *Styela montereyensis*, a stalked ascidian, (Young and Braithwaite, 1980), ambient flow augments active pumping. Stewart (2000) found that flow through chimneys of *M. membranacea* depended on ambient flow speed. This suggests the hypothesis that changes in the ambient flow environment might lead to remodeling of fluid transport systems that have openings onto the external environment.

Injuries to the colony would be expected to reduce flow to neighboring chimneys since injuries reduce the number of lophophores pumping fluid under the canopy to the chimneys. In addition, injuries can form new excurrent sites (Dick, 1987), which would be expected to further reduce flow to the existing chimneys. This suggests the hypothesis that injury to the colony might induce changes in the size or spacing of nearby chimneys.

What stimuli might affect remodeling of the canopy?

Two stimuli have been hypothesized to affect remodeling of the canopy: pressure under the canopy and injury (Dick, 1987). In *M. membranacea* and other bryozoans, chimneys have been observed at sites such as injuries where the canopy has been disrupted (Cook, 1977; Dick, 1987). Dick hypothesized that excurrent flow through the gap in the canopy at injured sites induces the surrounding lophophores to orient away from the site of the injury (Dick, 1987), thereby forming a chimney.

Because chimneys act to reduce the pressure under the canopy of lophophores (Grünbaum, 1995), it has been suggested that high pressure within the colony may induce chimney formation (Dick, 1987; Larsen and Riisgard, 2001). This hypothesis predicts that chimneys form within the canopy and not at the canopy edge because the canopy edge acts as an excurrent site where the pressure is low (Dick, 1987). Fluid flows under the canopy to exit the colony either at the chimneys or at the canopy edge. Since fluid flows from high-pressure sites to low-pressure sites, this indicates that both the chimneys and the canopy edge are at lower pressure than sites within the canopy (Grünbaum, 1995; Larsen and Riisgard, 2001). Therefore, the formation of chimneys at the canopy edge would disprove the hypothesis that high pressure under the canopy induces chimney formation (Dick, 1987).

Previous authors have observed structures that they interpreted to be partially formed chimneys at the canopy

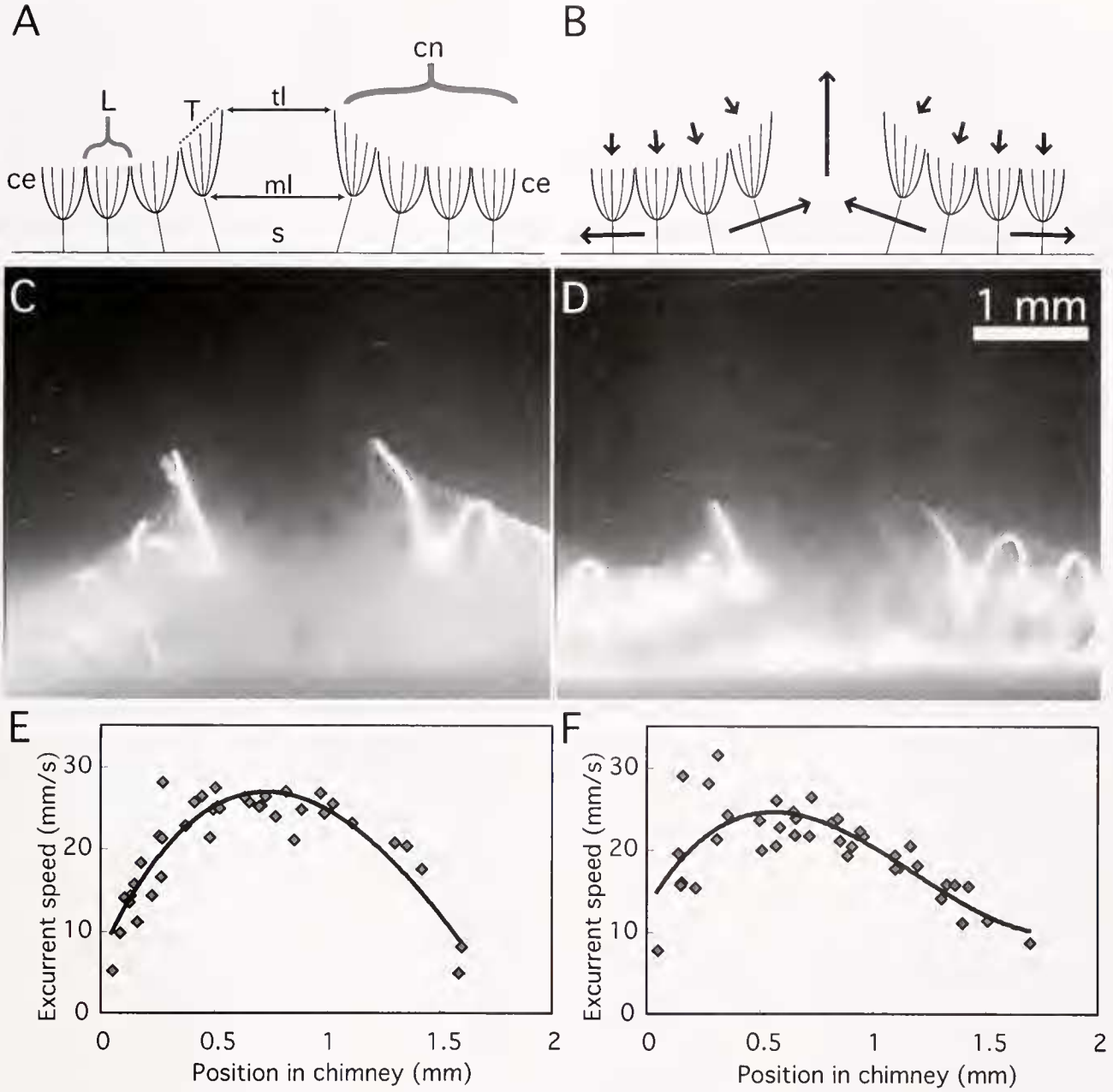

Figure 1. (A) A diagram of a *Membranipora membranacea* colony with a single chimney: canopy (cn), canopy edge (ce), lophophore (L), plane of tentacle tips (T) of a chimney lophophore, tentacle tip level (tl), and mouth level (ml) for the chimney, and colony surface (s). (B) A diagram of flow through the colony. Arrows indicate directions of flow. Small arrows indicate flow into lophophores. (C) A chimney in slow ambient flow. (D) A chimney in fast ambient flow. Colonies were illuminated with a laser sheet perpendicular to the colony surface and viewed from the side to show chimneys in cross section. Flow was to the left in both images. (E, F) Flow speeds out chimneys *versus* the distance from the downstream edge of the chimney ($x = 0$) along the line connecting the chimney edges: (E) slow ambient flow (chimney in C); (F) fast ambient flow (chimney in D).

edge (Cook and Chimonides, 1980; Dick, 1987). However, they did not observe whether these structures in fact became chimneys or just disappeared. To test whether chimneys form where the pressure under the canopy is highest, it is necessary to follow their formation through time to see where they form with respect to the canopy edge.

The goal of this study was to determine whether factors related to the environment—including ambient flow speed,

injury to the colony, or the pressure under the canopy—affect remodeling in the external fluid transport system of colonies of *M. membranacea*. I tested whether either ambient flow speed or injury to the colony influences the size or spacing of fully formed chimneys. I tested the hypothesis that new chimneys form at sites of high pressure under the canopy by observing where chimneys formed relative to excurrent sites. Finally, I tested the hypothesis that injury is sufficient to induce chimney formation by injuring groups of zooids of different sizes.

Materials and Methods

Colony collection

Colonies of *Membranipora membranacea* growing on laminarian kelp blades were collected off the Friday Harbor Laboratory dock, in Friday Harbor, Washington. Some previous studies have described the *Membranipora* species at Friday Harbor as *M. serrilamella* or *M. villosa* (Lidgard, 1981; Dick, 1987). However, recent studies indicate that these different morphotypes are the result of phenotypic plasticity for the presence of spines (Yoshioka, 1982; Harvell, 1984), and that all of the *Membranipora* colonies at Friday Harbor belong to a single interbreeding population (Schwaninger, 1999). I follow Harvell (1984) and Grünbaum (1997) in referring to colonies collected at Friday Harbor as *M. membranacea*. However, Schwaninger (1999) found significant genetic differentiation between the Friday Harbor population and the Atlantic population studied by Cook (1977), Cook and Chimonides (1980), and Okamura and Partridge (1999).

Ambient flow experiment: flow tanks

I built four flow tanks out of clear acrylic. The flow tanks were 2.3 cm wide, 5.5 cm tall, and 40 cm long. Pieces of acrylic were clipped to the tops of the tanks to close them. Dap silicon-rubber sealant was used to make a gasket around the top of the tank, and vacuum grease was spread on top of the gasket to make a tight seal. Hex-cell flow straighteners were placed towards the upstream end of the tanks. The working section was from 16.5 to 20.5 cm downstream from the flow straighteners. To maintain constant flow, the tanks were fed by a head tank and drained to a second tank. The head tank was supplied by seawater pumped from Friday Harbor.

Two flow treatments were used: the flow rate was 73.3 ± 0.5 ml/s in the fast-flow treatment, and 14.4 ± 0.2 ml/s in the slow-flow treatment. Slow flow was obtained using a single inflow tube—into which a plastic pipette tip with its end cut off was inserted—and a single outflow tube. Fast flow was obtained using two inflow and two outflow tubes (without the plastic pipette tips). This design allowed the tanks to be switched between the fast-flow and slow-flow treatments for different runs.

Colonies growing on flat pieces of kelp blades were collected. The pieces of kelp bearing the colonies were cut out and glued to pieces of plastic cut from a VWR brand weigh-boat using Duro "Quickgel" cyanoacrylate (superglue) gel. Colonies were selected that were between 3 cm^2 and 8 cm^2 in area, showed minimal damage, and were not bordered by neighboring colonies. One colony did have a very small colony (0.4 cm^2) growing next to it. Data from this colony fell within the range for the treatment and did not affect the results of any of the analyses. The colonies were placed in a sea-table with running seawater for 1 day prior to placement in the flow tanks.

A single colony, selected at random, was placed into each tank. All colonies were checked with a hand lens to remove any of the cryptic predatory nudibranch *Doridella steinbergae*. The plastic backings supporting the colonies were held onto the side of the tank by two strips of plastic cut from a weigh-boat mounted on the sides of the tanks. The colonies were held vertically to reduce fouling by debris. Seawater temperature ranged from 10 to 12 °C during the experiment.

Plan-view photographs were taken of the colonies in the flow tanks immediately before starting the flow, at about 10 min and 2 h after starting the flow, and once per day for 3 days subsequently. Photos were taken using a Nikon Coolpix 995 digital camera. A fiber optic illuminator was used for lighting in all but the first run, in which the camera flash was used.

Ambient flow experiment: flow measurements

To measure flow through the chimneys, one chimney in each colony was videotaped on the third day after the flow in the tank was started. Videos were made using a low-light, analog video camera (Watec 902A) with a macro lens. Chimneys were viewed from the side (*i.e.*, with the camera above the tank) to visualize excurrent flow (Fig. 1C, D). A red diode laser (World Star Tech) was used to make a sheet of light (<1 mm thick) that bisected the chimney. Particles naturally occurring in the seawater were used to visualize flow.

Chimneys were selected in which the lophophores were clearly visible but which were separated by no fewer than four lophophores from the canopy edge. It was difficult to get good images of chimneys in the middle of the colony because they were often obscured by chimneys closer to the colony edge. Since I could only visualize chimneys near the canopy edge and taping was done on the third day, chimneys used for these videos may not have been fully formed on the first day and therefore may not be the same ones used for measurements of chimney enlargement (see "Measurements of chimney enlargement" below). The flow rates

between these chimneys and chimneys closer to the colony center may differ, but there is no reason to expect that this would affect the shape of the flow profile within the chimneys or change the effects of ambient flow speed.

A PCI frame grabber (Scion LG3) was used to capture about 50 s of video from videotape onto a computer, where it was analyzed using NIH Image 1.62 software. Occasionally the lophophores would retract in the part of the colony in the field of view; those parts of the video were not analyzed. The video fields were separated to give 60 fields per second.

Particle streaks were selected that were bright, in focus, and intersected a line between the tentacle tips on the upstream and downstream edges of the chimney (see Fig. 1A). To ensure that the particles were visible throughout the entire 1/60 of a second covered by the field, only particles that were also visible in both the previous and subsequent fields were used. The x-y coordinates of either the beginnings or the ends of two consecutive streaks were measured to calculate particle velocities.

To calculate chimney diameter and to determine a chimney-centered coordinate system, I measured the x-y positions of the tentacle tips on the upstream and downstream edges of the chimneys. For each colony, 9 to 17 measurements were made at 3-s intervals.

The speed, the component of velocity out of the chimney (*i.e.*, normal to the colony), and the distance from the downstream edge of the chimney were calculated for each streak. I used Mathematica 3.0 to analyze data on particle streaks and tentacle-tip positions. Cubic polynomials were fitted to these data to calculate speed and velocity as a function of distance from the downstream chimney edge (Fig. 1E, F). Cubic polynomials were used because they appeared to fit the data well in both flow treatments. The maximum speed and the maximum component of velocity out of the chimney were calculated from these cubic polynomials. The relative position of maximum excurrent flow speed was measured as the distance between the downstream edge of the chimney and the site of the maximum excurrent flow speed divided by the diameter of the chimney.

To characterize the flow in the tank, imaging and streak measurements for the flow just upstream of the colony were made for one colony in the fast-flow treatment and one colony in the slow-flow treatment. The frame rate was 30 frames per second. The downstream component of velocity and the distance from the tank wall were calculated for each streak, using a computer spreadsheet. Shear rates—that is, the derivative (dU/dx) of flow velocity (U) with respect to distance (x) from a surface—were calculated from quadratic equations ($U = ax^2 + bx$) fitted to the data on particle velocities. Quadratic equations were used because laminar flow between parallel plates has a parabolic profile. Shear rates were 5 s^{-1} in the slow-flow treatment and 22 s^{-1} in the fast-flow treatment at the kelp surface, 2 mm upstream of the colony edge. These shear rates span much of the range of shear rates that colonies are likely to experience in the field (Grünbaum, 1997).

Injury experiment

To investigate whether injury to the colony affected the size or spacing of existing chimneys, and whether injury induced chimney formation, I observed the responses of colonies to injuries of different sizes. The treatments were "uninjured" controls (0 zooids killed), "4-zooid injuries" (4 zooids killed), "12-zooid injuries" (11 to 14 zooids killed), and "36-zooid injuries" (34 to 39 zooids killed). These treatments were chosen to span a range of sizes from that of typical chimneys (Lidgard, 1981) to injuries much larger than typical chimneys. I used a range of injury sizes because the local flow conditions at an injury and the flow to the neighboring chimneys are likely to depend on injury size, and because colonies in nature may receive injuries of different sizes.

I collected large colonies (>10 cm diameter) growing on flat pieces of kelp, and split the colonies into four or more pieces to form genetically identical colony fragments with intact growing edges. The pieces of algae were glued (using Duro "Quickgel" cyanoacrylate gel) to backings made from VWR-brand plastic weigh-boats. After 4 to 6 days in the sea-table, pieces from each parent colony were randomly assigned to each of the four injury treatments.

I injured patches of zooids that were located midway between three to four chimneys by breaking the zooid walls and frontal membrane with a needle. Injured zooids only rarely regenerated during the length of the experiment. Injured patches were roughly square. The colonies were then suspended vertically in the sea-tables with running seawater. Colonies were observed through a dissecting microscope and photographed in plan view before injury and at 3 and 11 days after injury.

To test whether the gaps in the canopy left by injuries were of a similar size to those produced by chimneys, I measured the area of the gaps in the canopy both at natural chimneys and at injuries. Measurements were made using images taken 3 days after injuring the colony pieces. I measured the gap area of the chimney nearest to the center of the image in each control colony piece. For both the injuries and the chimneys, I measured the area of the gaps in the canopy at the level of the tentacle tips (Fig. 1A) as the area of a polygon connecting the tentacle tips.

To test whether there was a difference in the posture of the zooids between chimneys and injuries, I calculated the "spreading ratio," the ratio of the actual area of the gap in the canopy to the expected area of the gap given the observed number of nonfeeding zooids. A spreading ratio greater than 1 indicates that the lophophores were held away

from the nonfeeding zooids, and a ratio less than 1 indicates that the lophophores were held over the nonfeeding zooids. The expected gap area was the number of nonfeeding zooids (in the injury or chimney) multiplied by the average area of non-chimney lophophores within each colony piece: it is an estimate of the total area of the lophophores removed. To determine the area of the non-chimney lophophores, I measured the area of two to three groups of 7 lophophores midway between pairs of chimneys in the same manner as I measured the gap area. In rare instances the injured zooids regenerated during the experiment, so the number of nonfeeding zooids differed slightly from the original number of injured zooids.

At 11 days, the morphology of zooids surrounding the injuries was observed using a dissecting microscope. I recorded whether the plane of the tentacle tips (Fig. 1A) of lophophores surrounding the injuries was parallel to the plane of the colony surface, tilted away from the injury, or tilted towards the injury. I also recorded whether the bases of lophophores surrounding the injuries were held higher than those of surrounding lophophores.

Measurements of chimney enlargement

I measured the enlargement in chimney area as the ratio of chimney area at time t to chimney area at time 0. I measured the enlargement in chimney spacing as the ratio of the distance between two chimneys at time t to the distance between them at time 0. Tentacle tips surrounding chimneys were sometimes difficult to see clearly in the plan-view photographs. Therefore, for measurements of chimney enlargement in both the injury and the ambient-flow experiment, I measured the area of chimneys at the level of the base of the chimney lophophores (about the level of the mouth, Fig. 1A). Chimney area and position were measured by drawing a polygon connecting the base of each of the lophophores bordering the chimney in a computer graphics program, and then measuring the area and x-y center of the polygon in NIH Image 1.62. Polygons formed by points on the colony skeleton were used as reference areas.

For the flow experiment described earlier, I measured the enlargement in chimney area and spacing from the time just prior to starting the flow to 3 days afterward. Because variation in enlargement in chimney area was high within individual colonies (see below), I calculated the median chimney enlargement of all the measurable chimneys in each colony. Colonies had between 1 and 13 (median of 5) measurable chimneys. I measured the enlargement in chimney spacing for a pair of randomly selected chimneys (using a random number generator). To ensure that chimneys were fully developed, I only measured chimneys that were separated by more than three lophophores from the canopy edge prior to starting the flow.

For the injury experiment, I measured the enlargement in chimney area and spacing from the time just prior to injury to 3 days after injury. I calculated the enlargement in area of the chimney nearest to the injury, and I calculated the enlargement in the spacing between that chimney and a second chimney close to the injury since these would be most influenced by the presence of the injury.

Measurements of chimney formation

Chimney formation was observed in colonies during the ambient flow experiment. Chimneys were selected that were completely surrounded by feeding zooids on day 3, but for which there was no sign of chimney formation on day 0. In the first image in which the chimney was visible, I measured whether the chimney was completely surrounded by lophophores and how many lophophores separated it from the canopy edge. If the lophophores were held away from the site where the chimney subsequently appeared—so that there was an indentation in the canopy edge—I scored the chimney as appearing at the canopy edge (0 lophophores from the canopy edge). If there was no sign of the chimney or of an indentation in the canopy edge, I scored the chimney as absent. I did not count chimneys in which there was an indentation in the canopy edge on day 0, since I did not want to overestimate the number of chimneys first appearing at the canopy edge relative to the number first appearing within the canopy.

Statistics and graphs

Nonparametric statistics were used because of the small sample size in all experiments. All tests were two-tailed. Most statistical tests were done using StatView 5.0. I used Mathematica 3.0 to calculate statistics for the squared ranks test, a nonparametric test for differences in variance between two independent samples (Conover, 1999). The Friedman test is a nonparametric test for comparing treatments with the data grouped into blocks (Conover, 1999), as in the injury experiment in this study in which all the pieces from the same parent colony represent a block. I implemented the method described in Conover (1999) for all comparisons of individual treatments to each other after the Friedman test. All box plots show the median, 1st and 3rd quartile, 1st and 9th decile, and minimum and maximum.

Results

Does ambient flow speed affect flow through chimneys?

Ambient flow speed affected the flow profile in the chimneys. The flow profile was nearly symmetrical in the slow-flow treatment (Fig. 1E) but not in the fast-flow treatment (Fig. 1F). Consistent with this difference in symmetry, the relative position of the maximum excurrent flow speed was significantly farther downstream of the center of the chimney in the fast-flow treatment than in the slow-flow treat-

ment (Fig. 2A; $P = 0.0002$, Mann-Whitney U test; $n = 10$ for each treatment). The maximum excurrent flow speed did not differ significantly between the two treatments (Fig. 2B; $P = 0.9$, Mann-Whitney U test), but the maximum of the component of velocity out of the chimney was significantly lower in the fast-flow treatment (Fig. 2C; $P = 0.002$, Mann-Whitney U test).

Does ambient flow speed affect chimney size and spacing?

The enlargement in chimney areas was calculated as the ratio of the chimney area at 3 days after starting the flow in the tank to the chimney area just before starting the flow. The variation in enlargement in chimney areas within individual colonies was high. The standard deviation in chimney enlargement was 23% ($n = 13$ chimneys) and 24% ($n = 11$ chimneys) for one colony in the fast-flow treatment and one colony in the slow-flow treatment.

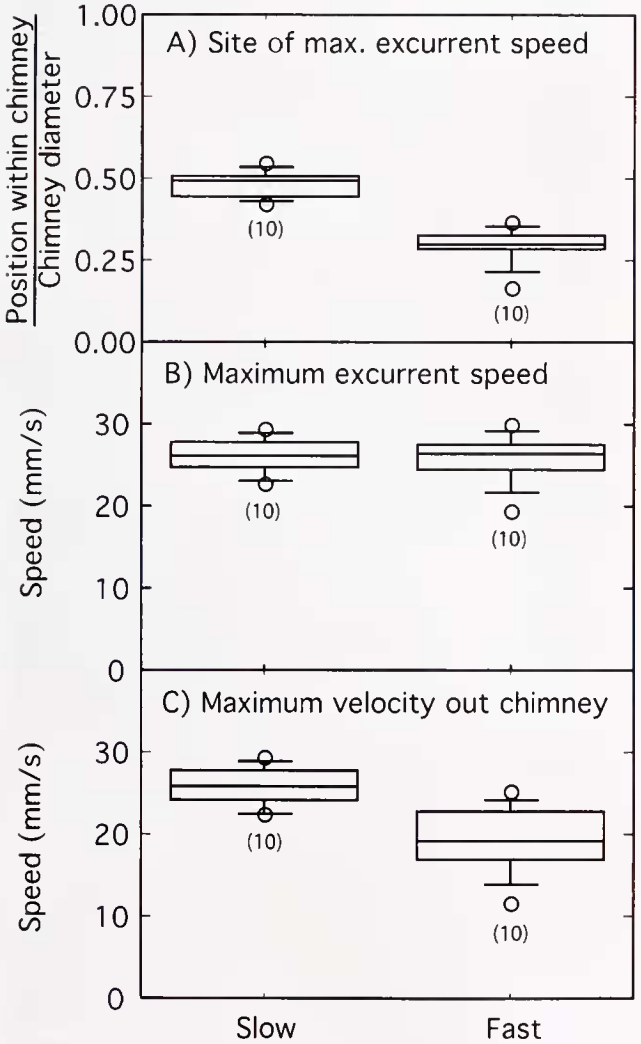

Figure 2. Effects of ambient flow speed on flow within chimneys. (A) The relative position of the site of maximum excurrent flow speed. The downstream side of the chimney is at $y = 0$, and the upstream side is at $y = 1$. The chimney center is at $y = 0.5$. (B) The maximum excurrent flow speed within the chimney in fast and slow ambient flow. (C) The maximum component of velocity out of the chimney in fast and slow ambient flow. Numbers of colonies measured are in parentheses. Box plots show the median, 1st and 3rd quartile, 1st and 9th decile, and minimum and maximum (open circles).

To test for an effect of ambient flow speed on the enlargement in chimney area, I calculated the median of the enlargement in chimney area for each colony (Fig. 3A). Chimney-area enlargement was significantly greater than 0% in the fast-flow treatment ($P = 0.005$, Wilcoxon signed-rank test; $n = 10$) but not in the slow-flow treatment ($P = 0.07$, Wilcoxon signed-rank test; $n = 9$). However, the difference in chimney-area enlargement between treatments was not statistically significant ($P = 0.09$, Mann-Whitney U test).

To test for an effect of ambient flow speed on the enlargement in chimney spacing, I measured the ratio of the distance between two randomly selected chimneys at 3 days after starting the flow in the tank to the distance between them just before starting the flow (Fig. 3B). One colony had only one measurable chimney, so that colony was excluded. The change in spacing was significantly greater than 0% in the slow-flow treatment ($P = 0.04$, Wilcoxon signed-rank test; $n = 9$), but not in the fast-flow treatment ($P = 0.1$, Wilcoxon signed-rank test; $n = 9$). However, the difference between treatments in the change in spacing was not statistically significant ($P = 0.7$, Mann-Whitney U test). The standard deviation of the change in chimney spacing was significantly greater in the fast-flow treatment (6.3%, $n = 9$) than in the slow-flow treatment (2.3%, $n = 9$; $P < 0.02$, squared-ranks test).

Where do chimneys form?

The hypothesis that high pressure under the canopy of lophophores induces chimney formation predicts that chimneys form within the canopy and not at the canopy edge (Dick, 1987). New chimneys appeared in most of the colonies in both treatments, and the process of chimney formation could be observed in photographs taken daily (Fig. 4). About half of the chimneys first appeared at the canopy edge, and no chimneys first appeared more than one lophophore from the canopy edge (Table 1).

Does injury affect nearby chimneys?

To test whether injury to the colony affected the morphology of nearby chimneys, I measured the enlargement in area and spacing for chimneys near to injuries of different sizes and in uninjured controls. None of the injury treatments differed significantly from each other or from the controls in the enlargement in either area or spacing (Fig. 5; $P = 0.6$ and $P = 1$ for area and spacing respectively, Friedman test; $n = 6$ blocks).

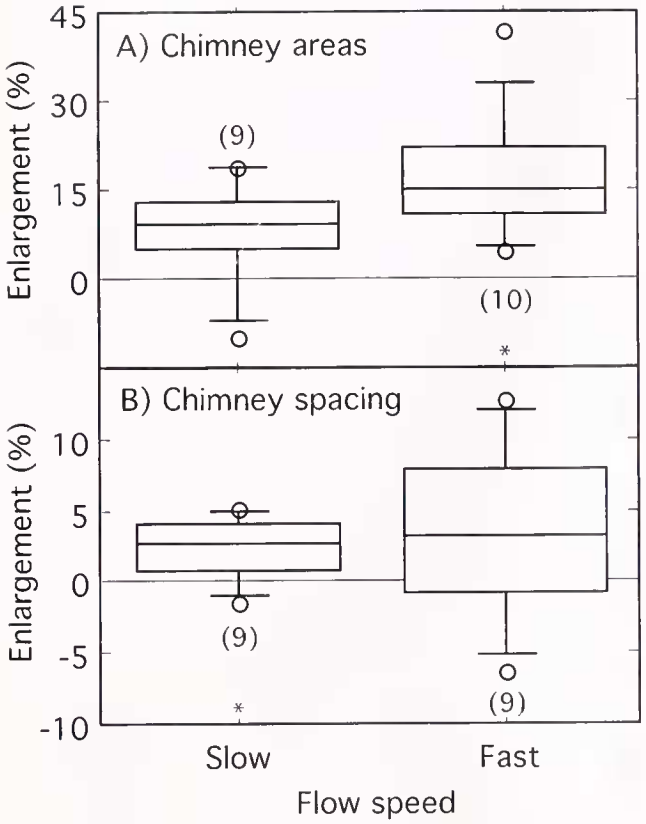

Figure 3. Changes in chimney area and spacing in fast and slow ambient flow. (A) Median enlargement in chimney area. (B) Enlargement in chimney spacing. Enlargement was significantly different from 0% for groups marked with an asterisk (*) ($P < 0.05$, Wilcoxon signed-rank test). Numbers of colonies measured are in parentheses. Box plots as in Figure 2.

Chimney areas increased significantly in all treatments (Fig. 5A; $P < 0.05$ for all treatments, Wilcoxon signed-rank tests; $n = 7$ to 8). Chimney spacing tended to increase in all treatments, but the increase was statistically significant in only one treatment, the 4-zooid injury treatment (Fig. 5B; $P = 0.03$, Wilcoxon signed-rank test; $n = 7$). The increase in spacing was not statistically significant in the other three injury treatments ($P \geq 0.3$ for all treatments, Wilcoxon signed-rank tests; $n = 7$ to 8).

Does injury induce chimney formation?

I observed the zooids bordering injured sites of different sizes to determine whether injury induces chimney formation. In normal chimneys, the stalks supporting the lophophores lean away from the chimneys so that the lophophores are held away from the chimney center, thereby forming an opening (Fig. 6A). In contrast, in every injury of all size classes, the stalks supporting the lophophores tilted towards the injured sites after 3 days, so that the lophophores surrounding the injury were held over the injury and closed or partially closed the gap in the canopy formed by the injury (Fig. 6B).

The spreading ratio (the ratio of the area of the gap in the canopy to the expected area of the gap given the number of nonfeeding zooids) provides an index of the extent to which the lophophores are held over or away from the nonfeeding zooids. There were significant differences in the spreading ratio between treatments (Fig. 6C; $P = 0.0007$, Friedman

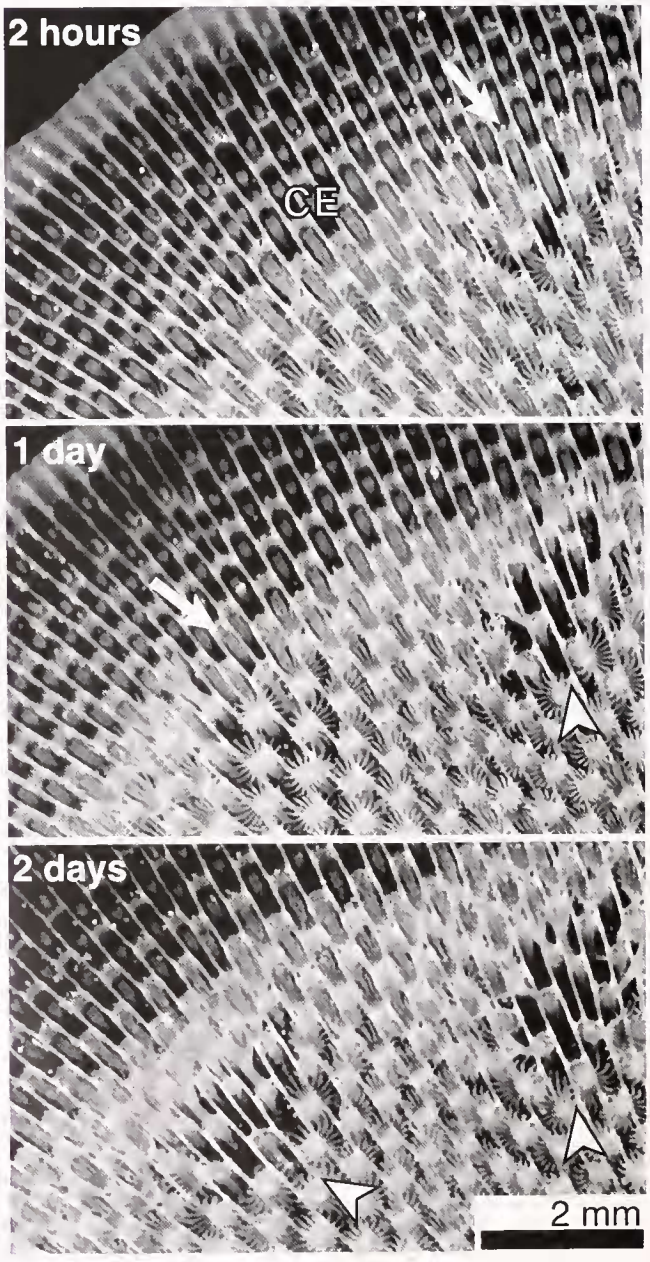

Figure 4. A time series of two chimneys forming at the canopy edge. Times shown are from when the flow was started in the tank. Canopy edge (CE), places where the lophophores spread apart to make an indentation in the canopy edge (arrows), fully formed chimneys (arrowheads). The colony edge is to the upper left.

Table 1

Numbers of chimneys formed at different distances from the canopy edge

	Separation from canopy edge (in lophophores)		
Flow treatment	0	1	≥2
Slow flow*	6	3	0
Fast flow**	11	11	0

* Slow-flow treatment: 9 chimneys formed in 5 colonies.
** Fast-flow treatment: 22 chimneys formed in 9 colonies.

test; $n = 6$ blocks). The spreading ratio for the chimneys was significantly larger than the spreading ratios for all size classes of injuries (Fig. 6C; $P < 0.05$, for all comparisons following the Friedman test; $n = 6$ blocks).

There were significant differences in the area of the gaps in the canopy between treatments (Fig. 6D; $P = 0.0005$, Friedman test; $n = 6$ blocks). The gap area at the chimneys was larger than the gap areas at injuries of all size classes (Fig. 6D). In addition, the gap area at the 36-zooid injuries was significantly larger than the gap area at both the 12-zooid and 4-zooid injuries, and the gap area at the 12-zooid injuries was significantly larger than the gap area at the 4-zooid injuries (Fig. 6D). All differences between treatments were statistically significant ($P < 0.05$, $n = 6$ blocks) in comparisons between treatments after the Friedman test. The number of nonfeeding zooids in the chimneys ranged from 1 to 14, with a median of 10 ($n = 6$).

Two other features of normal chimneys are that the planes of the tentacle tips of the chimney lophophores are tilted away from the chimney center, and the chimney lophophores are held higher than the lophophores of surrounding zooids (Fig. 1; Banta *et al.*, 1974; Lidgard, 1981). The orientation of the plane of the tentacle tips (Fig. 1A) of the lophophores surrounding the injuries was observed at 11 days in four of the sets of colony pieces. The planes of the tentacle tips of those lophophores surrounding large injuries were tilted away from the gap in the canopy left by the injury, but the planes of the tentacle tips of lophophores surrounding small injuries were held horizontally (Table 2). Injury size had a significant effect on the orientation of the plane of tentacle tips ($P = 0.04$, Friedman test; $n = 4$ blocks). There was no sign that the lophophores surrounding the injuries were held higher than the neighboring lophophores in any of the treatments.

Discussion

Does ambient flow or injury affect existing chimneys?

Flow may affect the formation of new chimneys (Grünbaum, 1997; Okamura and Partridge, 1999) but appeared to have little effect on the size and location of chimneys *after* they had formed in this study. The size of pre-existing chimneys increased significantly in several treatments in this study, suggesting that the chimneys have the capacity to enlarge after they have formed. However, neither ambient flow speed nor injury size had statistically significant effects on the magnitude of enlargement in area or in spacing of existing chimneys. Ambient flow speed did have a small but statistically significant effect on the variation in the enlargement in chimney spacing, with higher variation in faster ambient flow.

The length of time used in this study was sufficient for new chimneys to form and for old chimneys to enlarge

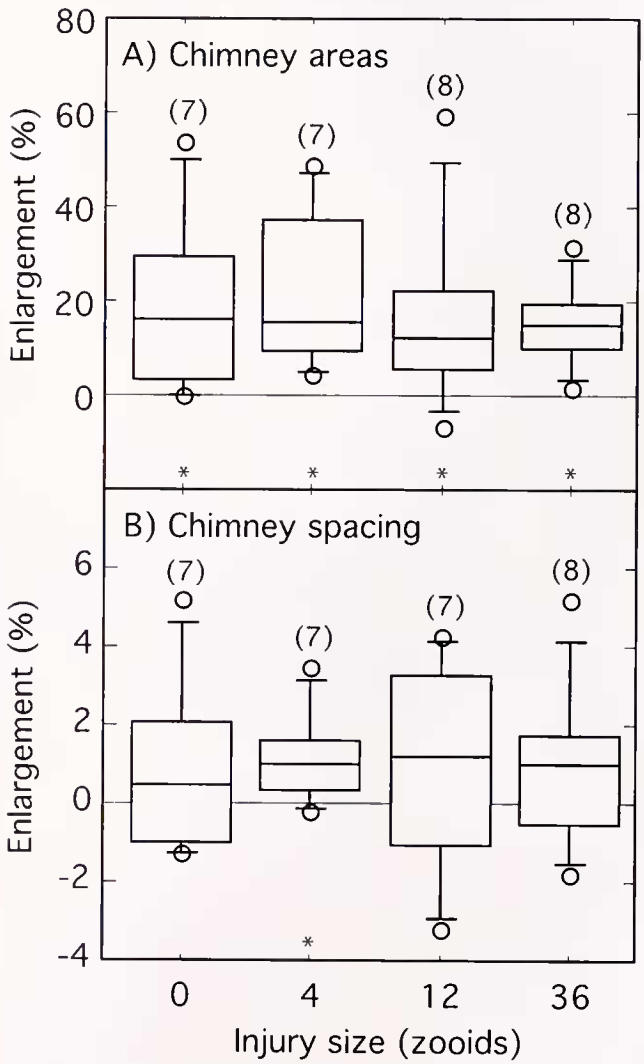

Figure 5. Changes in chimney area and spacing near injuries of different sizes. (A) Enlargement in area of individual chimneys. (B) Enlargement in chimney spacing. Enlargement was significantly different from 0% for groups marked with an asterisk (*) ($P < 0.05$, Wilcoxon signed-rank test). Numbers of colonies measured shown in parentheses. Box plots as in Figure 2.

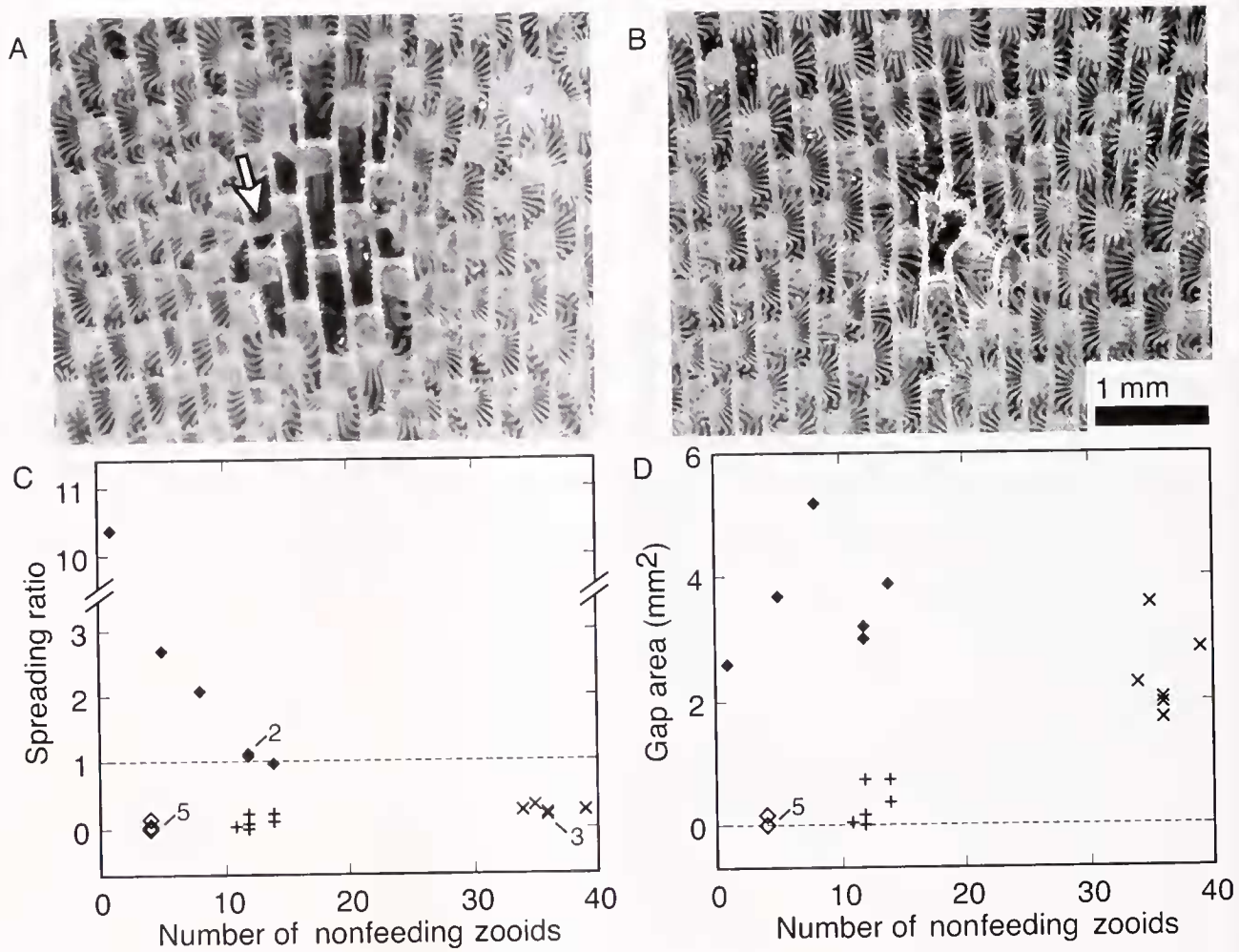

Figure 6. (A) A normal chimney with a single nonfeeding zooid viewed from above. The arrow points to a tentacle sheath (the stalk that supports the lophophore) that tilts away from the chimney center. (B) Zooids around a patch of 12 zooids that were injured 3 days before the photo was taken. (C) The spreading ratio *versus* the number of nonfeeding zooids for either chimneys or 3-day-old injuries. (D) The size of the gap in the canopy *versus* the number of nonfeeding zooids at either chimneys or 3-day-old injuries. Chimneys (filled diamonds); 4-zooid injuries (open diamonds); 12-zooid injuries (crosses); 36-zooid injuries (Xs); $n = 6$ for all treatments; numbers on plots indicate the number of overlapping values.

Table 2

Orientation of planes of tentacle tips on day 11

	Injury size (no. of zooids)		
Orientation	4	12	36
All horizontal	4	2	0
Mixed	0	1	0
All away from gap	0	1	4

Numbers shown are numbers of colonies in each category (4 colonies per treatment). Underlined treatments were not significantly different from each other ($P > 0.05$, after the Friedman test; $n = 4$ blocks).

significantly. This suggests that there was ample time for remodeling of chimneys.

Although I found little effect of ambient flow speed on the magnitude of the change in spacing of *existing* chimneys, previous studies on *Membranipora membranacea* suggest that ambient flow speed affects the spacing of the chimneys as they form (Okamura and Partridge, 1999). Okamura and Partridge (1999) found that chimney spacing depended on the ambient flow velocity at the sites in which colonies had grown in the field. In contrast, Grünbaum (1997) did not find that chimney spacing depended on the ambient flow velocity for colonies grown in flow tanks. However, he used lower flow speeds than colonies are likely to experience in the field.

The ambient flow conditions used in this study were within the range expected in the field. Grünbaum (1997) estimated that *M. membranipora* experiences shear rates from 3.5 to 45.7 s^{-1} in the field. The shear rates used in this study bracketed much of that range (from 5 to 22 s^{-1}). Grünbaum (1997) used lower shear rates (0.17 to 1.9 s^{-1}). Unfortunately, there is insufficient information to estimate the shear rates experienced by colonies in Okamura and Partridge's (1999) study.

Ambient flow speed did affect the flow profile, though not the maximum excurrent flow speed, within *M. membranacea* chimneys. Flow profiles within chimneys were clearly asymmetrical at high ambient flow speeds, but nearly symmetrical at low ambient flow speeds. In addition, the component of velocity out the chimney was lower in the treatment with high ambient flow speed than in the one with low ambient flow speed. The peak excurrent flow speeds were similar between the high and low ambient flow speeds. In contrast, previous studies done at lower ambient flow speeds than those used in this study found that excurrent flow speed increased with increasing ambient flow speed in *M. membranacea* (Stewart, 2000).

Effects of flow within the colony

Flow *within* the colony may also be important for determining where new chimneys form (Dick, 1987; Grünbaum, 1997). The odor of certain predatory nudibranchs induces the formation of defensive spines on the colony surface (Harvell, 1984; Grünbaum, 1997). These spines are expected to increase the resistance to flow under the canopy (Grünbaum, 1997). Chimney spacing is greater in colonies without spines than it is in colonies that have been induced to form spines as they grow, suggesting that new chimney spacing depends on the resistance to flow under the canopy (Grünbaum, 1997).

In contrast, I found no effect of injury on the size or spacing of the pre-existing chimneys near the injury. I expected injuries to reduce flow to neighboring chimneys both by removing lophophores that pumped fluid to those chimneys and by forming new openings in the canopy. This suggests that, in contrast to *new* chimneys (Grünbaum, 1997), the size and spacing of *existing* chimneys may not be affected by flow within the colony.

Pressure and chimney formation

Chimneys function to reduce the pressure under the canopy, so it has been hypothesized that they form where the pressure is highest (Dick, 1987; Larsen and Riisgard, 2001). Reducing the pressure under the canopy is expected to be important for *M. membranacea* colonies because high pressure is predicted to inhibit feeding by reducing the incurrent flow rate (Grünbaum, 1995; Larsen and Riisgard, 2001). Note that Pratt (2004) did not find a difference in the incurrent flow rate between isolated zooids and groups of eight zooids; however, hydrodynamic models suggest that the pressure effect would be more important for larger colonies (Grünbaum, 1995).

Two different hydrodynamic models suggest that the canopy edge should be a site of relatively low pressure because it is an excurrent site and fluid flows from high pressure to low pressure (Grünbaum, 1995; Larsen and Riisgard, 2001). Therefore, the hypothesis that high pressure under the canopy induces chimney formation predicts that chimneys should form within the canopy, and not at the canopy edge (Dick, 1987).

In this study, all newly formed chimneys started out at or very near the canopy edge, not within the canopy, indicating that chimneys do not form at sites of high pressure, but instead form at sites of low pressure. Dick (1987) and Cook and Chimonides (1980) observed indentations in the canopy edge that they interpreted as chimneys in the process of forming, but they did not observe whether these indentations subsequently became chimneys.

Injury and chimney formation

To explain his observation of chimneys at sites of damage to the colony, Dick (1987) hypothesized that excurrent flow at injured sites may induce the lophophores surrounding the injury to take on the tilted morphology of chimney lophophores.

In this study, injury did not induce chimney formation. Lophophores surrounding injuries closed over the gap in the canopy formed by the injury—the opposite of what one would find if injury induced chimney formation. However, there remained a gap in the canopy over large injuries. Lophophores surrounding these gaps tilted away from the gap, which is one of the characteristics of chimney lophophores, consistent with Dick's (1987) hypothesis. However, unlike the chimney lophophores, the lophophores around large injuries did not become noticeably taller than their neighbors. These observations suggest that injury is not sufficient to induce chimney formation.

An alternative hypothesis, that the canopy forms chimneys at sites of high excurrent flow but closes over sites of low excurrent flow, is consistent with the results of this study. This hypothesis is consistent both with the observation that chimneys form at the canopy edge (an excurrent site) and with the observation that only injuries large enough to leave a lasting opening in the canopy take on characteristics of chimneys. Many other hypotheses might also explain these observations.

Summary

I found few effects of environmental flow factors on the remodeling of the external fluid transport system of colonies

of *Membranipora membranacea*. Whereas existing chimneys tended to increase in area, neither ambient flow speed nor injury to the colony had statistically significant effects on the magnitude of the changes in the size and spacing of existing chimneys. New chimneys did not form either at sites of high pressure under the canopy or at sites of injuries. However, both the lophophores and the stalks supporting the lophophores changed orientation after injury to neighboring zooids, thereby closing or partially closing the gap in the canopy formed by the injury. This suggests that the canopy does have the capacity to remodel in response to injury. *New* chimney formation at the canopy edge appears to depend on environmental flow factors (Grünbaum, 1997; Okamura and Partridge, 1999), in contrast to my results on *existing* chimneys.

This study suggests that conduits in some fluid transport systems are capable of remodeling, but the extent of their remodeling may not be affected by changes in the flow through them. Previous studies on the effects of flow on the remodeling of conduits in biological fluid transport systems have focused on systems that are completely internal and are involved in the transport of fluids within the organism. Changing the flow through the system causes remodeling of existing conduits in the mammalian circulatory system (reviewed by LaBarbera, 1990, 1995; Langille, 1995), the gastrovascular system of hydroids (Dudgeon and Buss, 1996; Buss, 2001), and the veins of plasmodial slime molds (Nakagaki *et al.*, 2000, 2001). In contrast, I found little effect of changes in the flow on the extent of remodeling of existing conduits in *M. membranacea* colonies, though the flow does appear to affect the formation of new conduits (Grünbaum, 1997; Okamura and Partridge, 1999). The chimneys of *M. membranacea* form openings onto the ambient fluid that allow filtered water to leave the colony. It would be of interest to see whether changes in flow through conduits in other suspension-feeding systems affect the extent of remodeling of those conduits.

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